J. Agronomy & Crop Science **192**, 363—372 (2006)

ISSN 0931-2250

Crop/Stress Physiology

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Carbohydrate Profiles During Cotton Floral Bud (Square) Development

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With 6 figures

Received October 21, 2005; accepted March 31, 2006

Abstract

Cotton (*Gossypium hirsutum*) flower's showy corolla expands rapidly, then senesces quickly. Efficient opening of corollas is important for self- and cross-pollination, and indirectly lint yield. Carbohydrate relations are integral to bud water relations and growth, although they are not well understood. Soluble sugars and starch in developing floral buds and flowers of field-grown cotton plants were analysed. Buds contained significantly more sugar than starch. Sucrose and its hydrolysis products strongly contributed to increased sugars during corolla expansion, and contributed −0.211 MPa to osmotic potential. Water concentration was constant throughout expansion at 860 g kg^{-1} FW, suggesting sucrose import and hydrolysis are coordinated with other drivers of expansion. Floral sugar content declined sharply during senescence (69 % in 24 h). Although remobilization to other organs could partially explain this decline, we suggest the possibility that most sugar is broken down via respiration, resulting in the production of metabolic water and the significant 7 % increase in floral water concentration during senescence. In summary, imported sucrose and its hydrolysis products increased rapidly during cotton floral bud growth, and supported bud expansion by decreasing the water potential of bud tissue.

Key words: anthesis — carbohydrate composition — cotton — floral bud — plant growth and development — sucrose

Introduction

Cotton flowers are showy and ephemeral: rapid bud elongation leads to a large flower that often enters senescence in < 1 day. Cotton is both self- and cross-pollinated. The efficient opening of the large inflorescence in cotton fields where self-pollination is being encouraged is important because it promotes the placement of the anthers relative to the stigma such that plentiful amounts of the heavy sticky pollen can land on the stigma. This helps ensure that multiple seeds set in each carpel, thus providing a high potential lint yield (Poehlman 1977). Furthermore, corolla size has sometimes been related to pollinator attractiveness. Which pollinator attractiveness has sometimes been related to pollination efficiency, and pollination efficiency is often related to seed set. Thus the efficient opening of the large inflorescences could possibly benefit cross-pollination when desired.

The elongation rate of the floral bud increases during the last week preceding anthesis (Stewart 1986). This acceleration is thought to reflect a shift from cell-division activity to cell expansion, but the manner by which cell expansion in the cotton corolla is achieved is not known. A decrease in the cellular osmotic potential (increase in osmotic pressure) is probably involved because decreases in osmotic potential often indirectly contribute to cell expansion by decreasing the water potential in the cell. Water then moves into the cell down its potential gradient. The movement of water into the cell increases the cellular hydrostatic (turgor) pressure, which can then act as a driving force for cell expansion by pushing out against the cell wall matrix, which is somewhat elastic in growing cells such as these.

However, even if a decrease in cellular osmotic potential is involved, a number of questions relating to cotton floral bud expansion remain unanswered. For example, how is the osmotic potential decreased? If there is substantial accumulation of material, such as carbohydrate polymer that can be

broken down to provide an internal source of osmoticum during rapid expansion, then are there other contributors to cell expansion, such as relaxation of the cell wall? What processes are occurring in the bud after bloom and during early senescence, and are these consequential to processes affecting cell expansion in bud elongation?

The water used by, or passing through, the cotton floral bud is obtained primarily from the phloem because the floral bud has a higher water potential than that of the surrounding tissue (Trolinder et al. 1993). Thus water movement in the xylem would be out of the floral buds to the neighbouring leaves or meristematic tissues (Trolinder et al. 1993). The water potential of the phloem, in contrast, can include a large hydrostatic pressure initially developed by loading of sucrose and other solutes into the phloem in source tissues (van Bel 2003). The phloem is thus capable of supplying solution, and thus water, to the floral bud despite the presence of the relatively high water potential in the floral buds. The supply of water to the floral bud primarily via the phloem has been demonstrated for flowers of a number of species (Chapotin et al. 2003).

This flow in the phloem is largely the hydrostatic pressure-driven movement of a sucrose-rich solution. With the possible exception of potassium, other solutes are typically present at a much lower concentration than sucrose in the phloem. Potassium can be present at up to three-fourths the solute potential of sucrose in the phloem of some plants (Lang 1983). If we are to understand the osmoticum/carbohydrate dynamics of the cotton floral bud, then we especially need to understand the fates of sucrose in the growing floral bud.

Conceptually the carbohydrate relationships of the cotton floral buds should be dominated by different kinds of processes at different stages of development. For example, during early bud development, regulation of the rate of expansion is probably necessary. In contrast, during anthesis rapid expansion needs to be achieved. After anthesis, the flower or plant might be able to recover some of the costs of flowering, for example, by recycling carbohydrates for use in other parts of the plant.

During the early phases of bud expansion, some osmoticum is needed to help drive the expansion, but photoassimilate that is not used as osmoticum could be used to assist controlled expansion in various ways. For instance, the regulated formation and breakdown of carbohydrate polymers (e.g. starch) from sucrose could assist in the regulation of or buffering of the degree of osmotic potential present in the bud tissue cells. Starch accumulation has been shown to occur in floral buds of a number of species, possibly including the cotton floral bud during early expansion (Zhao and Oosterhuis 1998). Many of the species that possess starch-accumulating buds have non-ephemeral flowers that open and close several times over the course of several days and nights (Evans and Reid 1988, van Doorn and van Meeteren 2003). The cotton flower does not open again once it has closed, so the role of any starch accumulation in relation to the cotton floral bud expansion is not clear. Perhaps, the accumulation could possibly be regulating solute content in the bud and thus the rate of bud expansion. The ability to synthesize and accumulate starch could also possibly act as a means of buffering the rate of unloading. If active transport relays are present between axially adjoined sieve tubes (Thompson and Holbrook 2003), then sudden changes between loading and unloading rates could cause rapid changes in sucrose concentration in the phloem. These kinds of sudden changes might occur in a rapidly expanding floral bud that is primarily dependent on imported sucrose as an osmoticum source.

Metabolic respiration machinery is found in floral tissues because they are rapidly developing and growing organs. If this machinery was present at high levels, then the metabolic respiration could affect the carbohydrate balance of the tissues, which could lead to effects on the water relations of the tissues. This machinery, however, has not been documented to exist at high enough levels to have a major effect on the carbohydrate balance of growing floral buds. Nonetheless, respiratory breakdown of sucrose or its hydrolysis products could provide some non-reversible regulation of the osmotic potential in the growing floral buds.

Processes that increase the net effect of the hydrostatic pressure in the floral bud cells will tend to promote the rapid expansion that occurs during later stages of bud development. These processes would also need to be active during early bud expansion, and include factors that lead to an increase in the hydrostatic pressure, as well as those that decrease the needed amount of hydrostatic pressure to achieve a certain rate of expansion.

Processes that decrease the osmotic potential in the floral bud tissue, such as the hydrolysis of starch into oligosaccharides and glucose and the hydrolysis of sucrose into glucose and fructose,

would help attract water from the phloem or other surrounding tissues. The additional water would lead to an increase in hydrostatic pressure that would help drive expansion in floral bud cells (Salisbury and Ross 1978). Regulating the carbohydrate hydrolytic activity could thus provide a way of regulating the floral bud cell expansion. However, unless this regulation of hydrolytic activity is tied in with regulation of resynthesis of higher-order saccharides or a similar process, then it would effectively be regulation of the rate of decrease of the osmotic potential, not the direction of change in osmotic potential. Carbohydrates differ from many other osmotica in that larger-order saccharides can be hydrolysed to form smaller ones and thus decrease osmotic potential. During early anthesis of the flowers of several species, the cellular osmoticum increases rapidly. For example, in petals of the daylily (*Hemerocallis* spp.), which also has a showy ephemeral flower, rapid hydrolysis of fructan to hexose sugars provides such an increase in cellular osmoticum (Bieleski 1993). In rose (*Rosa hybrida*) petals, starch hydrolysis coincides with petal expansion, but does not appear to control it (Evans and Reid 1988). The cell expansion and water influx are possibly triggering starch hydrolysis to help maintain the osmotic potential needed for continued expansion of the rose petals. Similar mechanisms in which carbohydrate breakdown helps increase or maintain cellular osmoticum levels during rapid tissue elongation also occur in non-floral tissues. In sunflower (*Helianthus annuus*) hypocotyls, the hydrolysis of stored or imported sucrose increases cellular osmoticum and aids in an increase of cellular hydrostatic pressure or in the maintenance of osmotic potential during elongation (McNeil 1976). Sucrose hydrolysis also appears to contribute to floral stalk elongation in tulip (*Tulipa gesneriana*) (Lambrechts et al. 1994, Balk and de Boer 1999). Sucrose hydrolysis, although a possible contributor to floral-bud expansion in a number of species, has not been clearly documented in this capacity.

Processes that relieve the physical restraint in growth, such as a relaxation of the cell wall that is often seen during plant cell expansion, would allow an increase in cell volume and thus cause a decrease in solute concentration, and thus an increase in water potential. Such a relaxation has been suggested to occur in cotton floral bud expansion because neither the water potential nor the osmotic potential of cotton petals changes significantly from 3 days pre-anthesis to the day of anthesis (Trolinder et al. 1993). If an increase in soluble sugars is involved in cotton corolla expansion, the increase is likely to be regulated in coordination with other driving forces of expansion, such as cell wall extension. Cells, however, cannot enlarge indefinitely without additional deposition of cellular structural material. Because the rate of expansion is so rapid leading into cotton bud anthesis, we would anticipate that most cellular structural material would have been laid in place prior to this, in which case the expansion in anthesis would be inherently limited by the ability to maintain a hydrostatic pressure above a threshold value (Wei and Lintilhac 2003).

The cotton flower usually senesces on the same day of opening or no later than the next day. The soluble sugar remaining in the cotton corolla following anthesis could potentially be salvaged for use in other parts of the plant. During corolla senescence in daylily (Bieleski 1995) and carnation (*Dianthus caryophyllus*) (Nichols and Ho 1975), a substantial proportion of sugars in the petals can be salvaged through redistribution to other parts of the flower or plant. Similarly, Vergauwen et al. (2000) have suggested that the hexose sugars in *Campanula rapunculoides* L. petals, which are present in high concentration at anthesis because of rapid fructan hydrolysis in the petals, were probably exported before the petals completely wilted. If the senescing cotton corolla is a source of carbohydrate, then the nearby ovary could possibly use these exported sugars for growth or energy. In turn, this would have the potential to enhance fibre quality or yield by supporting early fibre elongation. Cotton fibre (individual epidermal cells on the outer integument of the seed coat) elongation begins on the day of anthesis (Stewart 1986), reaching more than 2.5 cm in length during the first 3 weeks after anthesis. On the other hand, if a preponderance of hydrolytic activity existed in the flower, then this might prevent the salvaging of the floral tissue carbohydrates for redistribution to other tissues during senescence. This is so because hexose sugars, the primary carbohydrate hydrolysis products, are not usually transported in phloem (Halaba and Rudnicki 1989). A high ratio of hydrolytic activity compared with synthetic activity might be present in a rapidly expanding ephemeral flower, such as the cotton floral bud in the late expansion stage (Stewart 1986).

If the carbohydrates are not substantially salvaged to other parts of the plant, then they are

possibly broken down through respiration with consequent production of metabolic water. This water would probably not be salvaged to the rest of the plant through the xylem because a discontinuity exists between the floral buds and the xylem in cotton (Trolinder et al. 1993). Water could possibly be salvaged by absorption into the phloem. If the water was salvaged, then it could potentially enhance fibre elongation. A strong relationship between cotton fibre elongation and fibre water content has been documented, albeit later in fibre development (Rabadia et al. 1999). Documentation of metabolic water production in plants is relatively rare compared to in animals, but if present in the senescing floral bud, this water production could have a noticeable effect on tissue water relations.

Various combinations of the processes described above could contribute to cotton floral bud elongation and later senescence. To assist in discriminating among these processes, this study sought to establish if the primary carbohydrate source of osmoticum during stages of development of the cotton floral bud was starch or sucrose, to determine the likelihood of substantial salvaging of floral bud solutes, and to subsequently draw some conclusions concerning the particular processes involved.

Materials and Methods

Plant material

Cotton seeds, cv. Deltapine 50 (Delta and Pine Land Co., Scott, MS, USA), were planted to approximately 10 plants per m row in a well-drained Marietta sandy loam with 1-m row spacing in 12 by 12-m plots at the Mississippi State University farm near Starkville (MS, USA). Nitrogen was applied at 5.6 g m^{-2} at planting, followed by 4.5 g m^{-2} midseason. Potassium was broadcast 35 days prior to planting at the rate of 6.7 g m^{-2}. Standard agricultural practices were followed for weed and insect control.

Floral buds at the first axillary position from the main stem, and similar in appearance, were tagged with small jeweller's tags on what was later determined to be 10-day pre-anthesis. The bud lengths and widths of a control set of 40 buds were recorded at 10, 7, 5, 3 and 0-days pre-anthesis. The dates of anthesis were also recorded. These data were used to determine the onset and progression of rapid bud elongation and the date of peak anthesis, and so by comparison, the days pre-anthesis of other similar, but destructively harvested, bud samples that had been tagged at the same time. These other tagged buds were harvested in midmorning at 10, 7, 5 and 3-days pre-anthesis, on the morning and afternoon of the day of anthesis, and at midmorning of the day after anthesis. The corollas were not open on the day after anthesis in this study. At the earlier stages, 16 buds were sampled for analysis. This number was gradually decreased to four buds/flowers sampled at the latest developmental stages. All buds were individually analysed for sugars and starch. The whole bud or flower was cut off with a razor blade below where the receptacle started to enlarge. The calyx was peeled off and discarded. The remaining bud or floral tissue was immediately and quickly diced with a razor blade, and then placed in pre-weighed microcentrifuge tubes containing small vent holes. The tubes containing the diced tissue were quickly plunged directly into liquid nitrogen, in which they remained for 1–3 months before extraction. At 2-days post-anthesis, the tissue was treated similarly, except that the ovary was handled separately from the rest of the tissue (which included the corolla, androecium and style).

Sugars and starch: extraction and analysis

The tissue stored in the microcentrifuge tube in liquid nitrogen was removed and plunged immediately into 75 °C 13.7 M (80 %) ethanol. The sugars were extracted by repeatedly incubating the tissue in the hot aqueous ethanol until bleached (Hendrix 1993). An excess of decolorizing carbon (C170–500; Fisher Scientific, Houston, TX, USA) was added to rid the aqueous ethanolic extracts of compounds that could interfere with enzyme activities. Aliquots of the extracts remaining from charcoal treatment were brought to near dryness, and then resolubilized in a known volume of water. These aqueous solutions were stored at −20 °C for < 30 days. The sugars (glucose, fructose and sucrose) in the aqueous solutions were quantified colorimetrically as β-nicotinamide adenine dinucleotide (reduced form) (NADH) at 340 nm. The NADH was generated stoichiometrically at room temperature through use of coupled-enzyme techniques (Hendrix and Peelen 1987). Glucose was assayed using half-strength Glucose Assay Reagent (G-3161; Sigma-Aldrich, St Louis, MO, USA), which utilizes hexokinase and glucose-6-phosphate dehydrogenase during NADH generation. After quantifying the NADH generated due to glucose, the reaction mixture in the cuvette was supplemented with 4.8 units of enzyme activity (under the conditions specified by the supplier) (EU) phosphoglucose isomerase (from *Bacillus stearothermophilus*) (P-5538; Sigma-Aldrich) per unit of glucose-6-phosphate dehydrogenase already present to allow additional NADH generation due to the quantity of fructose present. This enzyme was added at 0.6 % of the original assay volume, and was contained in 200 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid) (HEPES) buffered to pH 7.8. Finally, the reaction mixture was supplemented with 78 EU β-fructofuranosidase (from *Saccharomyces cerevisiae*) (Invertase I-9274; Sigma-Aldrich) per unit of glucose-6-phosphate dehydrogenase already present and the additional NADH generated due to sucrose presence determined. This enzyme was added at 3.9 % of the original assay volume, and was contained in 100 mM citrate buffered to pH 6.0. There was no indication of impurities in any of the enzyme preparations. The

standard curves for each sugar were highly significantly linear over the range of sample concentrations.

Starch in the residue left after sugar extraction was gelatinized by heating for 8 h at 80 °C in distilled water (Okechukwu et al. 1991, Yamamoto 1995). The gelatinized starch, in the equivalent of 100 mg dry weight or less of tissue, was digested at 55 °C for 2 h in 30 mM HEPES buffer (pH 6.9) with two additions of 50 EU glucoamylase from *Aspergillus niger* (amyloglucosidase A-3042; Sigma-Aldrich). This preparation of glucoamylase is free of contaminating enzyme activities that can hydrolyse structural carbohydrates and thus could have given falsely high readings for starch when assayed as glucose equivalents. An aliquot of the glucoamylase enzyme preparation was first desalted into reaction buffer by passages through four 5-ml Sephadex G-25 (Sigma-Aldrich) gel-filtration columns per ml of the stock glucoamylase preparation to remove glucose from the stock solution. Furthermore, an extra digestion step using additional glucoamylase did not increase the amount of recovered starch, thus indicating that the glucoamylase had exhaustively hydrolysed the starch that was present. The glucoamylase used was sufficient to digest starch standards of several-fold greater content, thus a sufficient amount of glucoamylase was added. The digested product was assayed as glucose using the procedures described above.

Statistical analysis

Transformations to account for proportional data and for lack of normality did not alter any results from the statistical analysis. Paired *t*-tests were used to evaluate the significance of differences between pairs of mean values at $P < 0.05$. The 95 % or 99 % CIs used in the figures allow easy visual estimation of the significance of differences between mean values (Cleveland 1985).

Results and Discussion

Floral bud growth

The elongation rate of the cotton floral buds increased after −7 to −5 days post-anthesis, whereas the increase in bud width remained linear with days to anthesis (Fig. 1). The increase in bud width is thought to reflect the cell-division activity involved in growth of the carpels, but the rapid increase in bud length is due to cell expansion in the corolla (Stewart 1986).

Non-structural carbohydrate composition during bud elongation

Starch was a minor fraction (i.e. significantly less than half) ($P < 0.05$) of the non-structural carbohydrates at all stages of bud development examined. Starch increased in concentration ($P < 0.05$) and content ($P < 0.05$) from −10 to 0 days post-anthesis (Fig. 2), while decreasing in proportion to the total non-structural carbohydrates. This indicates that starch polymerization, as a way of regulating osmotic potential-driven early cotton floral bud expansion, does not contribute substantially towards cotton corolla expansion in our study. Starch hydrolysis, as a way of providing

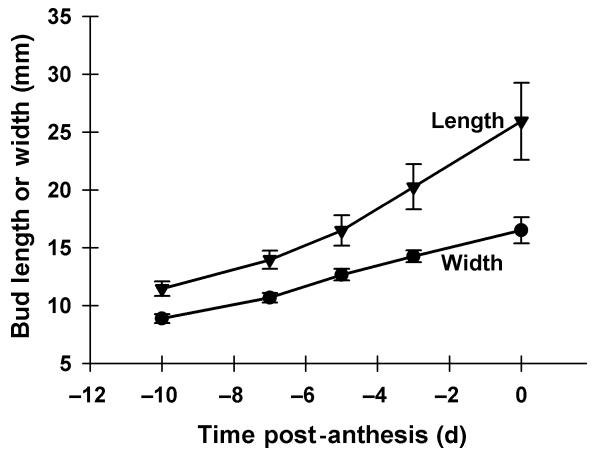

Fig. 1: Floral bud length and width from −10 to 0 days post-anthesis. The error bars are 99% CI. The increase in width (solid circles) is linear with days post-anthesis, but the rate of elongation (solid triangles) increases during the last 5–7 days before anthesis

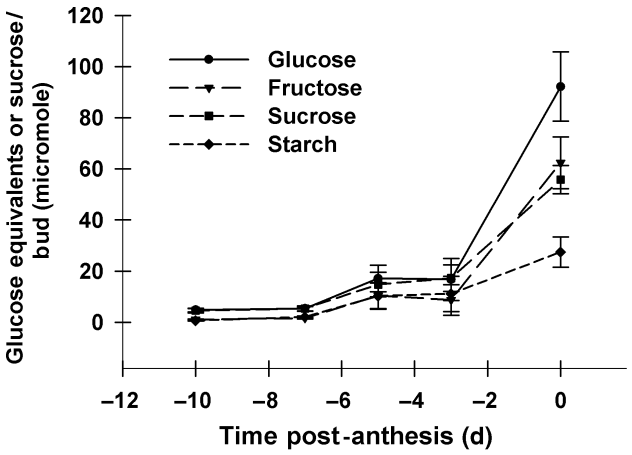

Fig. 2: Sugars and starch in pre-anthesis floral buds. The content and composition of glucose, fructose, sucrose and starch in floral buds and flowers from −10 to 0 days post-anthesis, expressed as micromole of sugar or starch (starch as glucose equivalents). The error bars are 95% CI. The soluble sugar content is much greater than the starch content, especially at anthesis when there is a sharp increase in content of these soluble sugars. The increases in glucose and fructose contents were especially pronounced

reserve osmotica during rapid bud expansion, also does not substantially contribute towards cotton corolla expansion in our study. In another study (Zhao and Oosterhuis 2000), starch constituted 87 % of the non-structural carbohydrate in cotton floral buds. These authors did observe, although, that a great increase in soluble sugar concentration accounted for most of a rapid increase in total non-structural carbohydrate concentration that occurred in the buds just prior to anthesis. Much of the increase in soluble sugar they observed appears to be due to sucrose and its hydrolysis products, based on the observed soluble sugar composition. Given the lower total non-structural carbohydrate concentration observed in their study (about half), a possible explanation of the differences in proportion of non-structural carbohydrates between the studies might lie in incomplete extraction of the sugars with consequent partial detection of the sugars as starch when assaying the starch as glucose equivalents. Otherwise, starch accumulation or hydrolysis could possibly contribute to cotton floral-bud cell expansion under other genotypic or environmental conditions than were present in our study. However, it is not imperative, as shown in our study, to have a substantial contribution by starch accumulation or hydrolysis towards cotton floral bud growth.

Sucrose hydrolysis appears to contribute to corolla expansion. During rapid bud elongation (i.e. −3 to 0 days), the soluble sugars (glucose, fructose and sucrose) increased in proportion relative to other solid constituents ($P < 0.05$) (Fig. 3). The increases in content, however, were greater for glucose ($P < 0.05$) and possibly fructose (not significant at $P < 0.05$) than for sucrose (Fig. 2). In situations such as this, when glucose and fructose increase in nearly equal amounts (1.3-fold more glucose in our case), then the hexose sugars are mostly the product of sucrose hydrolysis [e.g. β-fructofuranosidase (invertase) action] (Hendrix and Peelen 1987). The water concentration was constant during the period of rapid corolla expansion (Fig. 4), thus there was an increase in molality of these soluble sugars (calculated using the sugar content and water content of the entire bud, excluding calyx) associated with rapid bud elongation.

The increase in soluble sugar concentration of the bud was about half that observed in daylily petals, for which an increase in soluble sugar concentration due to fructan hydrolysis was shown to essentially drive petal expansion (Bieleski 1993).

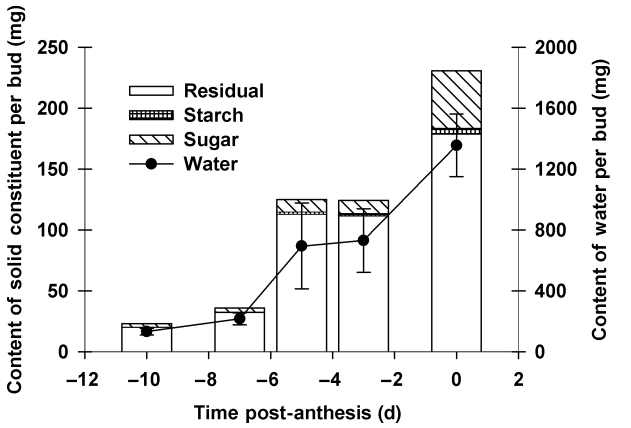

Fig. 3: Water and soluble sugars in pre-anthesis floral buds. The contents of water, soluble sugar (glucose, fructose and sucrose), starch and residue (mainly structural components) of floral buds and flowers at −10 to 0 days post-anthesis. The relative concentration increase was greater for soluble sugar than for the other constituents

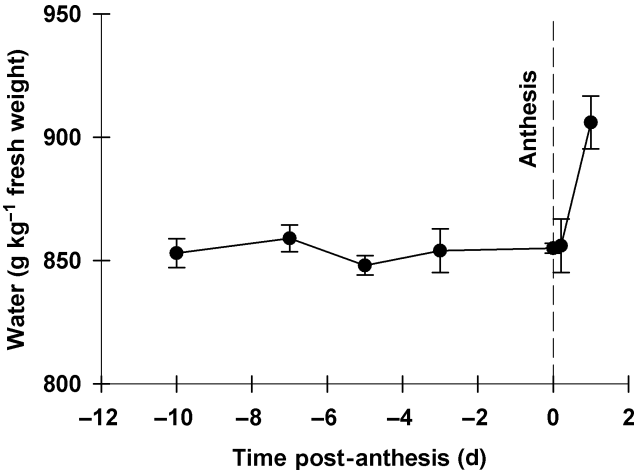

Fig. 4: Water concentration in floral buds, −10 to 1 days post-anthesis. The error bars are 95% CI. The concentration of fresh weight as water was constant through anthesis, and then increased after anthesis

In cotton, a more negative osmotic potential due to an increased soluble sugar concentration probably helps drive corolla expansion. Next, we estimated the extent of the contribution to osmotic potential. We know that the differences in activities of the hexose sugars compared with sucrose are minor at these concentrations (Peres and Macedo 1996), and we assumed that all of the increase in fructose content, and an equal amount of glucose, resulted from sucrose hydrolysis. We then calculated, using the Van't Hoff relation (Nobel 2005), the contributions of particular soluble sugar sources towards

the osmotic-potential change contributed by total soluble sugars between −3 and 0 days post-anthesis. Roughly 71 % was attributed to sucrose hydrolysis into glucose and fructose, 18 % to additional sucrose and 11 % to additional glucose. Thus, sucrose and its hydrolysis products could have contributed −0.211 MPa, enough for a 2.5-fold more negative partial osmotic potential due to these soluble sugars during the period of rapid corolla expansion. Sucrose hydrolysis in the cotton flower might be playing a role similar to that of fructan hydrolysis in the daylily flower, by acting to decrease osmotic potential and so helping drive corolla expansion. Other solutes, such as potassium and malate, are also likely contributors to a change in osmotic potential during corolla expansion (Stewart 1986), but do not have the capacity of larger-order saccharides to be hydrolysed into smaller osmotica and thereby decrease the solute potential.

The increases in concentrations of starch, sucrose, glucose and fructose in pre-anthesis development suggest that respiration-related breakdown of the sucrose is not non-reversibly regulating the decrease in osmotic potential in early bud development. These increases in concentration also suggest that carbohydrate polymerization is not reversibly regulating the solute potential through indirectly utilizing the imported sucrose.

The constant water concentration (Fig. 4), in the midst of other rapid changes associated with petal expansion, suggests coordinated regulation of cell wall relaxation with sucrose import and hydrolysis during petal expansion. This situation resembles that seen in rose-petal expansion in which starch hydrolysis appeared to be playing a role, but not a controlling one (Evans and Reid 1988). We suggest that in cotton corolla, sucrose hydrolysis can provide a large driving force in petal expansion. Some of the sucrose subjected to hydrolysis is recently imported. Starch hydrolysis possibly also contributes to this driving force under other conditions. Other factors, such as cell wall relaxation, are also coordinately involved in allowing the rapid corolla expansion.

Non-structural carbohydrate composition during floral senescence

The soluble sugar concentration (glucose, fructose and sucrose), but not the starch concentration (which was already low) of the flower declined sharply and significantly after full bloom, from

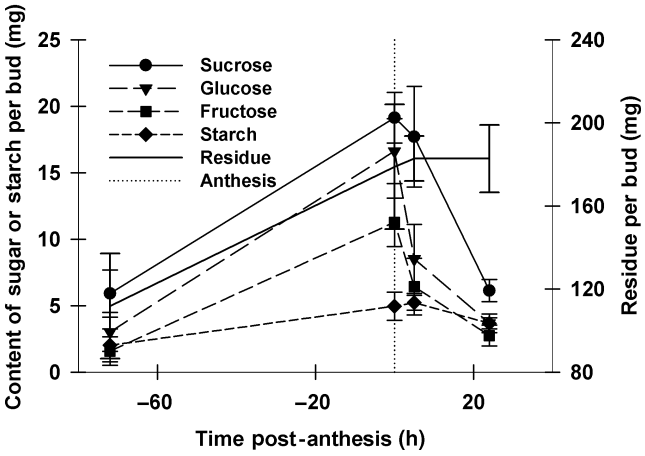

Fig. 5: Sugar and starch contents of floral buds during anthesis. The contents of sucrose, glucose, fructose, starch and residue (including structural components) in flowers or flower parts from −72 to 24 h post-anthesis. The error bars are 95% CI. There is an initial rapid loss of glucose and fructose from the flower post-anthesis followed by loss in sucrose along with that in glucose and fructose

205 g kg^{-1} dry weight down to 147 ($P < 0.05$) within 5 h, and further down to 64 ($P < 0.05$) within 24 h (Figs 5 and 6). The soluble sugars were low (2.5 ± 0.5 mg, 95 % CI) in 48 h corolla, androecium and style (i.e. the flower excluding the ovary) (Fig. 6). The senescing portion of the flower is not likely to be utilizing large amounts of

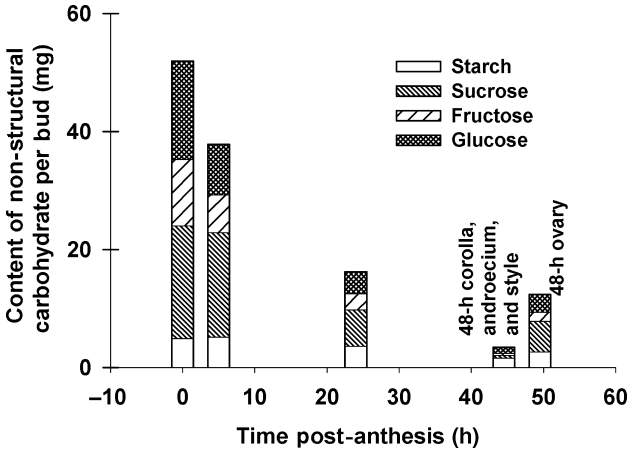

Fig. 6: Sugars and starch in post-anthesis floral buds. The contents of glucose, fructose, sucrose and starch in flowers from 0 to 48 h post-anthesis. At 48 h, the non-ovary tissue was separated from the ovary for analysis. The error bars are 95% CI. The soluble sugar (glucose, fructose plus sucrose) content of the non-ovary tissue (corolla, androecium and style) was significantly ($P < 0.05$) lower than in the developing ovary at 48 h post-anthesis

sugar for biosynthesis (because the content of non-structural carbohydrate plus residue is declining; Fig. 6) unless it is in support of salvage processes. The low level of sugar by 24 h probably results from redistribution to other plant parts (Nichols and Ho 1975, Bieleski 1995) or catabolism (Bieleski and Reid 1992). Nearly all (95 %) of the decline in sugars of the flower within the first 5 h post-anthesis occurs as a decline in glucose ($P < 0.05$) and fructose ($P < 0.05$) (Fig. 5). Sucrose is the form of transported sugar in the phloem, and the hexose sugars, relative to sucrose, can more directly enter into glycolysis and subsequently respiration. Thus, catabolism rather than export is more likely to explain sugar decline in this period. Another possibility is that sucrose export closely matches sucrose synthesis from hexose sugars during this period. However, this possibility seems unlikely because it would require an active phloem-loading process. During the period from 5 to 24 h after anthesis, sucrose declines ($P < 0.05$), like glucose ($P < 0.05$) and fructose ($P < 0.05$), to low levels, and sucrose export could help explain the sugar decline in the flower, although catabolism remains the simplest explanation.

The sugar concentrations are as great in 48 h ovaries as they were in 24 h whole flowers. Initiation of the cotton fibre occurs before anthesis, with elongation starting on the day of anthesis. The potential length of the cotton fibre is greatly influenced by events during the first few days post-anthesis (Stewart 1986). Secondary cell wall deposition, during which the bulk of the cellulose is deposited, occurs much later (16–19 days post-anthesis) (Schubert et al. 1973). Sugars salvaged from senescing floral material could potentially contribute to meeting the energy or substrate demands of the developing ovary or the new elongating fibres. Some transfer of sucrose from corolla to the ovary in senescing cotton flowers is possible. If the non-structural carbohydrate in the 5 h whole flower (38 ± 9 mg; 95 % CI) is all in petals and all available for export to the developing boll, then this could increase the dry weight of the young boll up to 30 %. In the more likely case that only the sucrose (approximately 18 mg) is available for export, then the potential dry weight increase drops to < 15 %. The mechanisms of phloem loading and transport impose further limits on the amount of sucrose available for export from the corolla, so we suggest that the contribution of salvaged corollar carbohydrates to ovary growth is not physiologically relevant.

The water concentration of the buds was relatively constant through 4 h post-anthesis, but then is sharply higher at 24 h post-anthesis (Fig. 4). This indicates that the post-anthesis flower is not wilting, but is rather senescing or undergoing another controlled change in state. Neither supply through xylem or phloem provides a ready explanation for this increase in water concentration. Xylem delivery would assume passage through a water potential compartment fairly near zero, and probably not in a serial gradient with the tracheary elements and the atmosphere. This contradicts the presence of the decreasing water-potential gradient from the flower to other neighbouring tissues that has previously been shown to exist earlier in development (Trolinder et al. 1993). Delivery through the phloem would require the disappearance of the accompanying sucrose. An alternate explanation is that mucilage is present and holding the water (Chapotin et al. 2003), although this begs the question of how the water content was increasing, because there is no evidence for an increase in residue content, which is the fraction that would contain mucilage. The simplest explanation is that the sugars in the floral tissue are not salvaged but are catabolized. The end-products of respiration are CO_2 and H_2O. For every 1.0 g of glucose broken down through respiration, 0.6 g of water is produced (Eckert and Randall 1983). Calculation shows that the amount of additional water content in the floral tissue closely matches what would be expected if respiratory breakdown was a major basis for the decrease in hexose sugar content in the flower after anthesis. Although the production of metabolic water is rarely documented in plants, this is the likely source of water here.

Conclusions

Sucrose hydrolysis products, but not starch hydrolysis, contributed to the partial osmotic potential due to soluble sugar during cotton corolla expansion, and probably helps drive the expansion. Cotton is one of the first species examined in which a breakdown of recently imported sucrose appears to be a major part of the mechanism in floral bud expansion. The potential contribution of salvaged corollar sugars towards the carbohydrate status of the young cotton ovary is small. The carbohydrates of the senescing flower are probably catabolized resulting in the production of metabolic water.

Acknowledgements

We are very grateful to Nicole E. Rafferty for her significant assistance in conducting this study.

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